The Effects of Irradiation on Plasma Proteins and Leukocytes in Thymectomized and Control Chickens* (33569)

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Recent research in avian immunology has done much to clarify the functions of primary lymphoid organs in birds. The bursa of Fabricius was shown to be responsible for the development of normal antibody producing ability of the chicken (1), while the avian thymus may function in the development of cellularly medicated reactions. Thus, while neonatal thymectomy impairs the development of allergic reactions (2) and tissue graft rejection ability (3 and 4), a function for the postneonatal avian thymus remains unknown.

In mammals, a postneonatal function for the thymus has been elucidated. This was accomplished by measuring immunological recovery from irradiation in thymectomized and control mice (5). The rate of this recovery can be used as an index of the function of the thymus. Since the avian thymus does not regress much during the early life of the bird (6), some function during this period of life seems likely. For this reason we decided to study recovery from irradiation in thymectomized and sham-operated chickens using changes in plasma proteins and numbers of peripheral WBC's as indices of recovery.

Materials and Methods. Fifty-two birds were used in this investigation. Twenty six of these birds were thymectomized at 2 weeks of age and the remainder were sham-thymectomized. At 8 weeks of age, half of the thymectomized and half of the sham-operated birds were irradiated with 750 R using Cobalt-60 as a gamma irradiation source.[2] The day of irradiation is designated day 0. On days 3, 6, and 10 blood samples were drawn from surviving birds for electrophoretic analysis. Electrophoresis was performed using the Beckman microzone electrophoresis system as described previously (7). In addition, blood smears were made and stained in Giemsa. The differential counts were made using a sample of 100 white blood cells, and the frequency distribution of small, medium, and large lymphocytes was obtained by micrometer measurement of cell diameters.

Results. Plasma protein changes. It is obvious from the day 3 values in Table I that a 40–50% drop in total plasma protein occurred in all birds due to the irradiation treatment. This drop was due to decreased amounts of albumin, alpha-2, beta-2, gamma-1, and gamma-2 globulin values. In addition, the thymectomized–irradiated group showed a significant depression in the beta-1 globulin value in comparison to the beta-1 value of the thymectomized–nonirradiated control group. The beta-1 value of the thymectomized–nonirradiated control birds was, however, initially higher than that of the sham-operated control birds, thus the depression of this value in the thymectomized irradiated birds was greater than when compared to the depression of the beta-1 value in the sham-irradiated group. The greatest changes induced by irradiation occurred in the albumin and gamma globulin protein fractions where over 50% reductions were measured.

Comparing the days 6 and 10 protein values with the day 3 values, one can see the pattern of recovery of the plasma protein fractions. By day 6 all of the globulins recovered to at least the original level and in most cases there was an overcompensation so that the recovery values were greater than the starting values. The albumin values, however, never returned to the magnitude of the starting values. By day 10, the high levels of the

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TABLE I. Absolute Plasma Protein Values in Irradiated and Control Thymectomized and Sham-Operated Chickens.[a]

Treatment	Days after irradiation	Total	Albumin	Globulins					
				Alpha-1	Alpha-2	Beta-1	Beta-2	Gamma-1	Gamma-2
Nonirradiated sham-operated	3	3.59 ± 0.53	1.52 ± 0.25	0.15 ± 0.05	0.18 ± 0.06	0.33 ± 0.07	0.38 ± 0.09	0.62 ± 0.24	0.42 ± 0.14
	6	3.78 ± 0.60	1.41 ± 0.19	0.23 ± 0.07	0.18 ± 0.05	0.34 ± 0.08	0.41 ± 0.05	0.70 ± 0.37	0.48 ± 0.17
	10								
Nonirradiated thymectomized	3	3.86 ± 0.46	1.52 ± 0.23	0.21 ± 0.09	0.21 ± 0.07	0.42 ± 0.10	0.45 ± 0.06	0.62 ± 0.19	0.39 ± 0.13
	6	4.06 ± 0.47	1.46 ± 0.34	0.23 ± 0.11	0.20 ± 0.06	0.41 ± 0.10	0.52 ± 0.12	0.71 ± 0.17	0.48 ± 0.14
	10								
Irradiated sham-operated	3	2.12 ± 0.24	0.79 ± 0.11	0.16 ± 0.03	0.14 ± 0.01	0.30 ± 0.07	0.23 ± 0.05	0.31 ± 0.05	0.19 ± 0.05
	6	4.26 ± 0.58	1.34 ± 0.21	0.31 ± 0.12	0.24 ± 0.09	0.44 ± 0.14	0.50 ± 0.15	0.81 ± 0.24	0.63 ± 0.17
	10	4.92 ± 1.03	1.02 ± 0.10	0.47 ± 0.08	0.21 ± 0.05	0.55 ± 0.06	0.62 ± 0.15	1.24 ± 0.50	0.81 ± 0.30
Irradiated thymectomized	3	2.02 ± 0.29	0.79 ± 0.13	0.15 ± 0.05	0.11 ± 0.03	0.25 ± 0.05	0.21 ± 0.05	0.32 ± 0.08	0.19 ± 0.06
	6	4.39 ± 0.87	1.14 ± 0.32	0.37 ± 0.09	0.33 ± 0.19	0.51 ± 0.19	0.53 ± 0.14	0.94 ± 0.21	0.63 ± 0.16
	10	5.49 ± 0.48	1.23 ± 0.41	0.48 ± 0.09	0.29 ± 0.03	0.69 ± 0.12	0.62 ± 0.15	1.43 ± 0.21	0.73 ± 0.18

[a] Means ± SD; grams % protein.

globulins were striking in both the thymectomized and the sham-operated irradiated birds. This increase was also reflected by the total plasma protein values for these two groups. The increases in gamma globulins were especially impressive. The day 10 gamma-1 globulin values were twice those of the control nonirradiated values. They were not significantly depressed immediately following irradiation but the values doubled by day 10 in both of the irradiated groups.

Differential counts. The data in Table II, illustrate that thymectomy caused a significant depression in the number of small lymphocytes in comparison to the numbers in sham-operated control birds. This depression is evident from day 3 as well as day 6 data. The reader must be reminded, however, that surgery was performed on these birds 6 weeks before these counts were taken; days 3 and 6 are used to indicate the number of days after irradiation treatment.

All lymphocyte types were markedly depressed in all irradiated birds 3 days after irradiation, illustrating their vulnerability to irradiation. These data show that there was a striking reduction in total number of lymphocytes, as well as a change in the ratio of WBC types. As a result, the ratio of granulocytes to lymphocytes was markedly elevated on day 3 in both irradiated groups. The day 3 medium lymphocyte value for the sham-irradiated group was significantly greater than that of the thymectomized irradiated group.

By day 6, lymphocyte populations in both groups had rebounded and as a result the ratio of granulocytes to lymphocytes decreased.

Discussion. The cause of the decrease in the plasma proteins concentrations following irradiation is unknown, but there are a number of possibilities. It is possible that the decrease may reflect blood loss which may have occurred as a result of irradiation damage. Petechial hemorrhages were noticed in the skin of some birds and histological examination of the intestine revealed the expected damage to the gut epithelium. Microscopic examination revealed the presence of protein

TABLE II. Frequency Distribution of Leukocytes in Peripheral Blood Smears from Thymectomized and Sham-Operated Chicken (irradiated and nonirradiated) (means ± SD).

Group	Days after irradiation	Small lymphocytes (6–8)	Medium lymphocytes (9–11)	Large lymphocytes (12–13)	Granulocytes
Nonirradiated	3	20.67 ± 16.95[a]	53.33 ± 15.68	6.83 ± 3.65	22.67 ± 15.01
sham-operated	6	35.39 ± 19.05[a]	43.02 ± 10.62	8.33 ± 3.02	17.61 ± 6.69
Nonirradiated	3	9.50 ± 7.07	55.93 ± 5.81	13.64 ± 6.96	22.93 ± 7.81
thymectomized	6	19.70 ± 12.40	38.70 ± 11.68	10.70 ± 4.11	33.01 ± 15.45
Irradiated	3	0	15.77 ± 12.18[b]	3.15 ± 2.16	79.92 ± 10.72[b]
sham-operated	6	25.02 ± 13.00	58.72 ± 36.61	6.80 ± 2.98	9.46 ± 3.33
Irradiated	3	0	7.82 ± 2.05	2.00 ± 2.19	90.18 ± 7.40
thymectomized	6	18.60 ± 8.51	50.40 ± 25.22	8.60 ± 3.86	24.90 ± 9.66

[a] Significantly different from the corresponding value in the thymectomized group.
[b] Significantly different from the corresponding value in the thymectomized–irradiated group.

plugs in the kidney tubules of some birds, suggesting probable kidney damage which could result in loss of plasma protein. One of the major initial changes in response to irradiation was a sharp drop in albumin, and the loss of albumin seems disproportionate in relation to the levels of other plasma proteins. Since the molecular size of albumin fraction is smaller than the other plasma protein fractions, there is a good possibility that the change reflects irradiation damage which directly (injury to membrane) and/or indirectly (induction of shock) affects the capillary permeability. It is also possible that the radiation altered the metabolic activity of the liver, altering the rate of synthesis of these proteins.

Regardless of the reason for the effect, it is important to follow the regeneration pattern. Plasma protein homeostasis was reestablished by day 6 and levels higher than day zero values were reached by day 10. The changes in alpha-1 globulin are striking. They did not decrease following irradiation, but demonstrated markedly elevated levels by day 10. These changes would indicate that the overall metabolic activity of the liver was stimulated and over-generation of all of the plasma globulins occurred. Albumin levels did not attain their original levels. This response may represent a compensation to maintain osmotic equilibrium in the face of the marked increase in globulin levels.

The marked rebound and high production of the gamma globulin in irradiated birds was of special interest. Theoretically the circulating gamma-globulin titer reflects the sum total of all circulating antibodies directed against several antigens. Does this indeed indicate an active, rebounding immune system, or does it indicate an overproduction of nonspecific, nonimmune gamma globulin by hepatic cells? Following irradiation the birds may have been exposed to substantial amount of antigenic material. Some of this antigenic material may have entered through the damaged digestive tract. Other antigenic material could result from tissue damage caused by the irradiation. This might stimulate a regenerating immune system, and the gamma globulin titer would thus rise to high levels. The data seem to support the hypothesis that the thymus is not important to the maintenance or production of plasma proteins.

Following irradiation, a precipitous drop in lymphocyte numbers occurred. No small lymphocytes were noticed in the circulation by day 3 following irradiation. However, by day 6 small lymphocyte numbers reappeared. Small lymphocyte numbers increased in both thymectomized and control groups. Medium sized lymphocyte frequencies were slow to rebound in the thymectomized group. This may indicate that the thymus continues to function in adding lymphocytes to the general circulation. Indeed, even the small lymphocyte data are suggestive of such an effect although the results are not statistically different from controls.

Summary. This experiment was conducted in an attempt to elucidate a postneonatal function for the thymus in chickens. Twenty-six chickens were thymectomized at 2 weeks of age and 26 were sham-operated. At 8 weeks of age all birds were irradiated with 750 R. Plasma protein and leukocyte values were measured in survivors. Three days after irradiation a 40–50% decrease in plasma protein occurred in control and thymectomized birds. This decrease was due to decreased amounts of albumin, and alpha-2, beta-2, and gamma globulin. By day 6 all of the globulins recovered to preirradiation levels, and in most cases there was an overcompensation noticeable by day 10. The albumin values, however, never returned to the magnitude of the starting values. No differences were observed between plasma protein responses of thymectomized and intact birds. Thymectomy caused a depression in the number of circulating small lymphocytes. The numbers of all lymphocyte types were markedly depressed by irradiation but they rebounded by day 6. Medium and small lymphocyte numbers did not recover as rapidly in thymectomized as in sham-operated birds. It is postulated that the postnatal avian thymus may contribute to the populations of small and medium lymphocytes.

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